Fate and effects of cadmium in near shore environments

interactions between Cd and the Lugworm, Arenicola marina

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FATE AND EFFECTS OF CADMIUM IN NEAR SHORE ENVIRONMENTS - INTERACTIONS BETWEEN Cd AND THE LUGWORM, *ARENICOLA MARINA*

Ph.D.-thesis

Department of Life Sciences and Chemistry
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25-11-98

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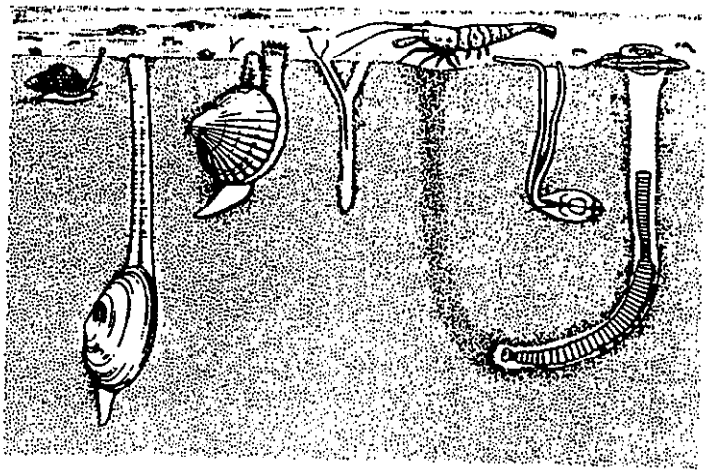
In this thesis, the abbreviation AWP means Apparent Water Permeability, a term introduced by Smith (1970) and described in the appendix. Osmoadaptation is defined as physiological, biochemical and behavioural processes in osmoconforming or osmoregulating invertebrates ensuring some degree of adaptation to the external osmolarity.

The roman numerals explained on page 19 are used for quoting articles (papers published/submitted/in press and research reports).



The *Macoma* community

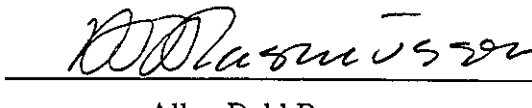
Preface and acknowledgements

This thesis is the result of my research during 3 years to receive the Danish Ph.D. degree in Natural Sciences. The experimental work was performed at the Department of Life Sciences and Chemistry, Roskilde University, Denmark. A stay at the Alfred Wegener Institute, University of Bremen, Germany, was very beneficial for increasing my general knowledge about the lugworm and for learning specific methods for measuring biochemical parameters in lugworms.

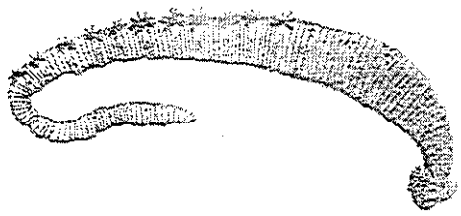
I would especially like to thank my supervisor Professor Dr. med. Ole Andersen for many good hours of inspiring discussions as well as for helping me out with several less science-related problems. Also, I would like to thank the German "crew" Dr. Doris Abele-Oeschger, Tanja, Katja and Petra for valuable assistance during my stay at the University of Bremen.

Special thanks go to Dr. Gary T. Banta and Dr. Valery Forbes, RUC, for valuable help and assistance during all phases of making this thesis and to Dr. Peter Holm, VKI, for help with the Minteq metal speciation model and for commenting on my work on Cd sorption to sediments.

Finally, I thank everybody at the department for help and advice.



Allan Dahl Rasmussen
Roskilde, November 1998



Arenicola marina - The lugworm

The beach/shore (Latin: arena) digger (Latin: colere) of marine areas

Abstract

Dansk

Skæbne og effekter af partikel-reaktive forurenende stoffer som for eksempel tungmetallet Cd er vigtige emneområder i økotoksikologien. Dette er særlig vigtigt for estuarine områder hvor Cd akkumuleres i sedimenter (Campbell & Tessier 1996) og udgør en fare for den estuarine biota (Viarengo 1985). Estuarier kan karakteriseres ved salinitetsændringer der influerer Cd's speciering Mantoura et al. 1978) og dermed Cd's skæbne og økotoksikologi (Rainbow 1995). Desuden vil bioturberende infauna påvirke optagelse og fordeling af Cd i sediment og dermed tilgængeligheden af Cd for infauna (Everaarts & SaralaDevi 1996). Omvendt kan metaleksponering påvirke bioturbationen (Miron et al. 1994). Slutteligt kan de osmo-regulerings/-adapterings mekanismer, estuarine invertebrater anvender til at klare salinitetsændringer, påvirkes af Cd (Rasmussen et al. 1995).

Formålet med denne afhandling var at undersøge flere relaterede spørgsmål:
Hvordan vil salinitet og den bioturberende organisme *Arenicola marina* påvirke fordelingen of Cd mellem vand og sediment? Er der andre vigtige estuarine faktorer der kan påvirke denne fordeling? Kan eksponering til Cd påvirke sandormens bioturbation?

Effekterne af salinitetsændringer modvirkes af *A. marina* ved hjælp af volumenregulering. Kan Cd påvirke denne regulering? Er dette toksisk for dyret?

De vigtigste resultater af undersøgelserne var:

i) Sandormens bioturbation øgede såvel sedimentets Cd-optagelse som den vertikale fordeling af Cd i sedimentet når Cd blev tilsat vandfasen (I). Dette blev underbygget af et mindre *in situ* eksperiment (IV). Denne kraftige omfordeling af Cd i sedimentsøjlen blev også observeret hvis et tyndt Cd-mærket sedimentlag blev placeret øverst på sedimentet eller hvis hele sedimetsøjlen blev homogent mærket med Cd (II). Det menes at bioturbation generelt øger mobiliseringen af Cd fra sediment til vand. Ikke desto mindre peger resultaterne på en netto Cd-fluks fra vand til sediment da sandormens stærke irrigation pumper det overliggende vand dybt ned i sedimentet hvor opløst Cd bindes (II). Selv om en salinitetsstigning fra 12 til 18 og 24‰ resulterede i en øget Cd frigivelse

blev denne effekt modvirker af sandormenes irrigation (II). Sedimenter virker derfor åbenbart mere som et Cd-lager end en Cd-kilde. Desuden blev sedimenternes optagelse og frigivelse af Cd påvirket af den salinitetsbestemte Cd-speciering samt af forholdet mellem sedimentets overfladeareal og vandets volumen. På grund af dannelsen af Cd-sediment komplekser vil bevægelsen af Cd fra vand til sediment stige med eksponeringstiden og faldende ratio mellem overfladeareal og vandvolumen. Følgeligt må opholdstiden for vandbåret Cd i et kystnært område påvirke bevægelsen af Cd fra vand til sediment (III). Ved høje Cd-koncentrationer blev optagelsesraten af Cd i sediment reduceret men den totale Cd-bevægelse blev øget på grund af den høje Cd-koncentration (I, III). Data fra en computerbaseret specierings-model foreslog at den fri hydratiserede Cd^{2+} ion var primært ansvarlig for bevægelsen af Cd fra vand til sediment (III).

Artklerne I → III beskriver udelukkende laboratorieeksperimenter. Relevansen af resultatene fra disse eksperimenter blev sat i perspektiv i en undersøgelse af den bioturberende effekt af en sandormepopulation i Roskilde fjord. Selv over små afstande kunne bioturbationen variere betragteligt fra praktisk taget intet til over 1 L sediment og 22 L vand flyttet af sandormene per kvadratmeter per dag ved de højeste populationsdensiteter (IV). Følgeligt må disse forskelle i bioturbationens intensitet kunne resultere i store lokale variationer i sedimenters optagelse, fordeling og frigivelse of Cd.

ii) Korttids eksponering (24 timer) til Cd hæmmede sandormenes evne til at regulere kropsvolumen, både når saliniteten blev holdt konstant og efter et fald i ekstern salinitet (V). Denne hæmning sås også efter langtidseksponering (1 måned) for Cd ved fast salinitet (V). Cadmiumeksponerede orm indeholdt mere cølomvæske end ikke-eksponerede orm, da den osmotisk drevne indfluks af vand ikke blev effektivt modvirket, sandsynligvis fordi Cd hæmmede processer involveret i urinproduktion. Volumenregulering er en del af ormens osmoadaptive strategi og den Cd-inducerede forstyrrelse af ormenes vand- og saltbalance kunne være ansvarlig for ormenes øgede mortalitet. Samtidig eksponering for antropogen og naturlig stress så derfor ud til at virke synergistisk (V). Den samme effekt sås i orm udsat for anoxi og lavere salinitet, det vil sige højere vandindhold i cølomet (VI). Hverken korttids Cd-eksponering eller anoxi påvirkede ormenes vandgennemtrængelighed (AWP, Apparent Water Permeability), men langtidseksponering til Cd havde en svag effekt på chloragogenvævets indhold af glykogen. Eksponering til Cd påvirkede også ormenes volumenregulering ved eksponering til øget salinitet (VI), men mekanismerne ansvarlige for dette er ikke klarlagte.

English

The fate and effects of particle reactive pollutants such as e.g. the toxic metal cadmium (Cd) is a subject at high research priority in marine ecotoxicology. This is particularly true for studies of estuaries because Cd accumulate in estuarine sediments (Campbell & Tessier 1996) and pose a threat to the estuarine biota (Viarengo 1985). Estuaries are characterised by salinity changes that will influence Cd speciation (Mantoura et al. 1978) and thus Cd fate and ecotoxicological impact (Rainbow 1995). Also, bioturbating infauna will affect the uptake and distribution of Cd in sediment, and thus bioavailability of Cd to infaunal organisms (Everaarts & SaralaDevi 1996). In contrast, metal exposure can affect bioturbation (Miron et al. 1994). Furthermore, the osmoadaptational processes developed in estuarine invertebrates as an adaptation to the salinity changes are compromised by Cd exposure (Rasmussen et al. 1995).

The purpose of this thesis was to investigate several interrelated questions:

How does salinity and the bioturbating organism *Arenicola marina* affect the distribution of Cd between water and sediments and are there other estuarine factors that could potentially influence this distribution? Will Cd exposure in turn affect lugworm bioturbation?

The effects of salinity changes are counteracted by *A. marina* by volume regulation. How will Cd exposure affect this regulation? Will this be potentially harmful to the animal?

The main results of the investigations were:

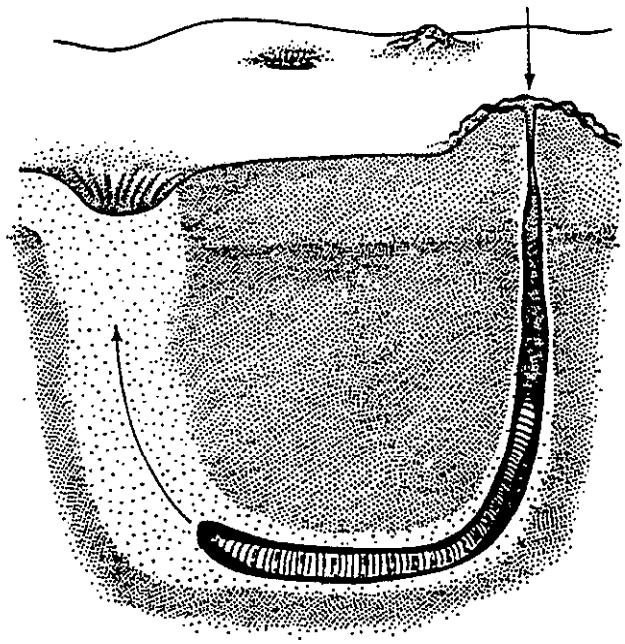
i) When Cd was added to the water phase, bioturbation by the lugworm increased both sediment uptake of Cd and vertical spreading of Cd in the sediment column (I). This was corroborated by results from a small *in situ* experiment (IV). Also, when a thin layer of Cd labelled sediment was placed on top of the sediment surface or when the entire sediment column was homogeneously labelled with Cd (II), lugworm bioturbation caused extensive redistribution of Cd within the sediment column. The general belief is that bioturbation will increase the mobilisation of sediment bound Cd back to the water phase. However, the results suggested a net Cd flux from water to sediment due to the strong irrigation of the lugworm (II) which pumps overlying water deep into the sediment where dissolved Cd is trapped. Even though an increased release of Cd from sediments occured when the salinity was increased from 12 to 18 or 24‰, this effect was counteracted by irrigation when lugworms were present (II). Sediments thus seemed to act mainly

as a Cd "sink" and not a Cd "source". Furthermore, sediment uptake and release of Cd was affected by the salinity-controlled speciation of Cd as well as by the ratio between sediment surface area and water volume. Due to the formation of Cd-sediment complexes, the transfer of Cd from water to sediment will increase with increasing time of exposure and decreasing ratio between the volume of Cd-laden water and the sediment surface area. Accordingly, the residence time of water-borne Cd in a shallow water system will affect the degree of Cd transfer to sediment (III). At high Cd concentrations the fractional uptake rate of Cd into sediment was reduced but there was an overall increase in total Cd transfer to sediment due to the high Cd concentrations (I, III). Data from a computer-based speciation model suggested that the free hydrated Cd^{2+} ion was the Cd species primarily responsible for Cd transfer to sediment (III).

Articles I → III exclusively describe laboratory experiments. The relevance of the results of these experiments were put into perspective by assessing the bioturbation of a lugworm population in Roskild Fjord. Even over small distances, lugworm bioturbation would vary tremendously ranging from virtually zero and to over 1 L of sediment and 22 L of seawater moved by lugworms per square metre per day at the highest population density (IV). Accordingly, these differences in bioturbation intensity could result in large local variations in sediment uptake, distribution and release of Cd.

ii) Short-term (24 hours) exposure to Cd inhibited the ability of lugworms to regulate their body volume when kept at constant salinitity or after reduction of external salinity (V). Also, long-term (1 month) Cd exposure inhibited the volume regulatory ability at constant salinitity (V). Cadmium-exposed worms contained larger volumes of coelomic fluid than unexposed worms because the osmotically driven influx of water was not effectively counteracted, most likely due to Cd inhibition of some process involved in urine production. Volume regulation is part of the worm's osmoadaptational strategy, and the Cd-induced disturbance of water and solute balance could be responsible for the observed increased mortality in worms. Simultaneous exposure to natural and anthropogenic stress factors thus seemed to act synergistically (V). Exposure of worms to anoxia prior to reduced salinity resulted in a similar effect, i.e., higher water content in the coelomic cavity and increased mortality (VI). Neither short-term Cd exposure nor anoxia affected lugworm Apparent Water Permeability (AWP), but long-term Cd exposure had a slight effect on glycogen content of chloragogenous tissue. Cadmium exposure also affected the volume regulatory

response of lugworms during exposure to increased external salinity (VI), but the nature of this effect needs further investigations.



The lugworm in it´s burrow

The arrows indicate the direction of irrigational water movement

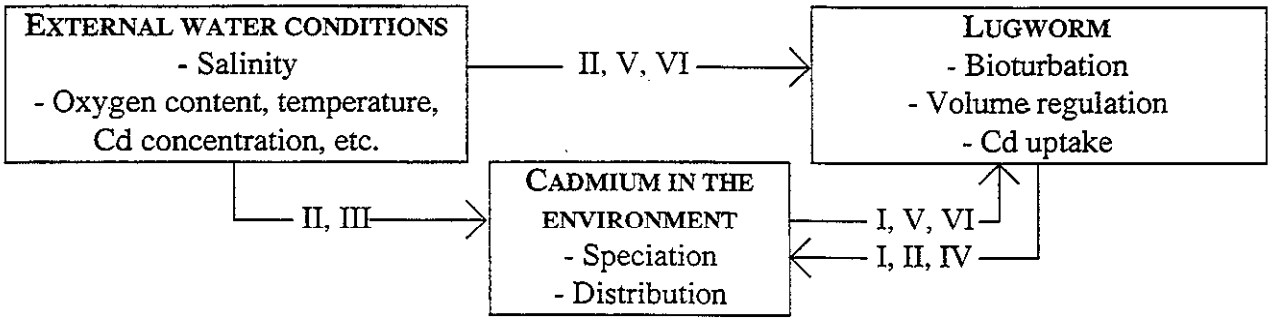
Aim of the present study

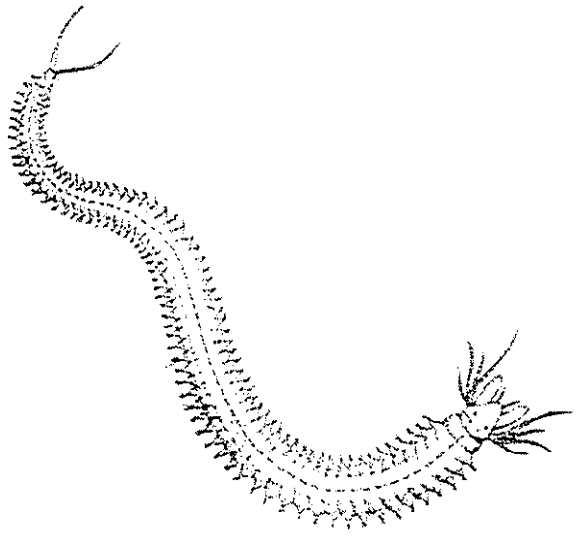
Salinity changes frequently occuring in estuarine areas affect the distribution and toxicity of the particle reactive and toxic metal Cd in several ways. Thus, salinity is the main factor controlling the speciation of Cd in seawater (Kester 1986). Also, estuaries may receive and retain Cd from urban, industrial and rural areas (Campbell & Tessier 1996) and from athmospheric fallout (Jensen & Markussen 1993). The resulting sediment Cd concentrations may be very high, especially in shallow areas (Lalli & Parsons 1993). Consequently, uptake, distribution and effects of Cd have been investigated in much detail in benthic invertebrates (Viarengo 1985, Simkiss & Taylor 1989). Infaunal organisms may strongly affect several processes at the sediment-water interface due to bioturbation (Krantzberg 1985), thereby changing sediment uptake and release of Cd and ultimately their own exposure to Cd (Everaarts & SaralaDevi 1996). Furthermore, the changing estuarine environment demands that estuarine organisms possess osmoregulatory and osmoadaptational capabilities that enable them to buffer their internal milieu against external salinity changes (Rankin & Davenport 1981). These capabilities have been demonstrated to be succeptible to Cd exposure (Rasmussen et al. 1995).

To study the complex interactions between infauna, toxicants and fluctuating salinities in estuaries described above, a simplified laboratory system was employed: Bioturbation and salinity were selected as main experimental parameters affecting the distribution of Cd between water, sediment and infauna. The lugworm (*Arenicola marina*) was chosen as model organism as it is a strong bioturbator which effectively modifyes several processes at the sediment-water interface and within sediment (Krantzberg 1985). The lugworm species (*Arenicola* and *Abarenicola* spp.) process sediment in a conveyor-belt fashion and are distributed world-wide (Wells 1963). The results obtained from this work are thus of general interest. As the lugworm is a slightly hyper-osmotic osmoconformer (Reitze & Schöttler 1989), using volume regulation to adapt to salinity changes, volume regulation was used as an indicator of physiological performance. The combination of salinity changes as a natural stress factor and Cd exposure as an anthropogenic stress factor was used to study Cd toxicity under conditions more relevant to natural habitats than those usually used in toxicity tests which are typically performed with stable external conditions. Also, measurements of lugworm AWP were

included as this osmoregulatory parameter was shown to be affected in the shore crab *Carcinus maenas* by Cd exposure (Rasmussen et al. 1995) and because it could potentially contribute to an understanding of volume regulation.

An overview of the relationships investigated. The roman numerals refer to the papers and reports listed on page 19





Nereis diversicolor

Another common polychaete found at St. Havelse

Introduction

The lugworm *Arenicola marina* (Derived from Kershaw 1984, Brusca & Brusca 1990, Dorit et al. 1991, Knight-Jones et al. 1995).

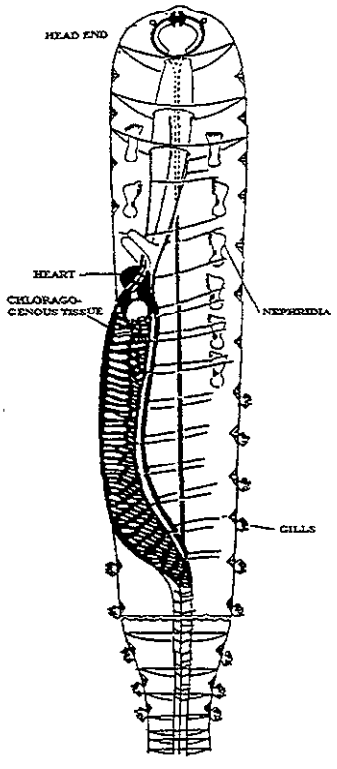
Taxonomy, morphology and physiology

Phylum	Annelida.
Class	Polychaeta
Order	Capitellida
Family	Arenicolidae
Brusca & Brusca, 1990	

The annelidae comprises about 15.000 species of segmented worms and has successfully invaded all humid habitats. The name of the phylum originates from the greek "annulatus", meaning "ringed". The phylum concists of the classes polychaeta (meaning "many-chetae", e.g. *Arenicola*), oligochaeta (few chetae, e.g. eartsworms) and hirudinida (leeches). Although usually used as a synomenon for *Arenicola marina*, the term "lugworm" comprise the two genera *Arenicola* and *Abarenicola* as well as two worm genera without specialised tails. These four genera together constitute the family Arenicolidae (Wells, 1963). In the present text *Arenicola marina* and "the lugworm" will be used interchangeably.

Some of the general characteristics of the annelidae are:

- bilateral symmetri and segmention.
- digestive tract complete, usually with regional specialization.
- closed circulatory system.
- well developed nervous system.
- most possess metanephridia, less commonly, protonephridia.
- paired segmentally arranged epidermal setae bundles.
- head composed of presegmental prostomium and peristomium.
- Gonochoristic or hermaphroditic.
- both marine, terrestrial, and freshwater species exist.



Some of the special characteristics of *A. marina* are:

- The coelom is not compartmentalized except by three transverse septae (barriers) at the anterior end, and the longitudinal and circular muscles are organized as two cylinders outside the

coelomic compartments. A segment (metamere) is constitued by one coelomic compartment and the surrounding body wall. This can be recognized externally by the transverse grooves in the integument. Neither the head or prostomium anterior to the mouth, nor the terminal part of the body, the pygidium, which carries the anus, is a segment.

- The main part - the trunk - of the body consists of 19 segments, all carrying setae. The last 13 segments have gills.
- The segmentation makes a localized hydrostatic skeleton possible.
- The cuticle covering the body is made of scleroprotein or mucopolysaccharide fibres.

Food enters the foregut, moves to the midgut where digestion occurs, and eventually reaches the hindgut and anus. Digestion is predominantly extracellular. Connected with the gut is the chloragogenous tissue that acts as a combined liver and storage tissue. The body of *Arenicola* consists of about 90% water, which is mainly coelomic fluid. Coelomic fluid is slightly hyperosmotic compared to the external medium thereby supplying a hydrostatic skeleton used for peristaltic movement. The coelomic fluid further functions in excretion of waste products, maturing gametes, and as a general circulatory system. Waste products as well as gametes are excreted via the metanephridia that are connected to the coelomic fluid.

Distribution & mode of life

The European lugworm is found in intertidal and near-shore sediments composed of clean to muddy sand on North-west European coasts, from the Arctic to the Mediterranean (Knight-Jones et al. 1995). Members of the arenicolidae family are found in suitable near-coastal sediments throughout the world (Wells 1963).

Arenicola marina is a sedentary infaunal annelid. It lives in a fixed L- or U-shaped burrow for long periods of time (Krüger 1971). One end of the burrow is open so that the lugworm can crawl up backwards and defaecate. This defaecation happens every 5 to 60 minuttes, depending on lugworm size and eating activity, and leaves the characteristic sand-casts on top of the sediment (Wells 1966). The head is situated at the other (deep) end of the burrow. Here the worm feeds on the sediment in front of it. The depth of the burrow depends on the size/age of the animal - older animals living deeper in the sediment (Krüger 1971). The deeper sediment is usually anoxic, but the lugworm ventilates its burrow by rythmic wave-like movements of the body thereby drawing freshly oxygenated water down

the back-end of the burrow, past the body where the gills are situated, eventually ejecting it at the head end (Riisgård & Banta in press). Besides supplying oxygen for the metabolism of the worm this loosens the sediment in front of the worm, making it easier to eat, and at the same time perhaps promote a "garden of bacteria" (Hylleberg 1975) that the worm feeds.

Osmoregulation and osmoconforming

The physics

The tendency of a pure solvent to enter a solution separated from it by a semipermeable membrane is termed osmosis (Atkins 1989). An example of this could be the water flow through cell membranes. As the chemical potential of the solvent will tend to be the same at each side of the membrane at equilibrium, the osmotic pressure (Π) can be calculated as:

$$\Pi = [B]*R*T$$

where [B]= the concentration of the solute (mol), R = the gas constant ($J*K^{-1}*mol^{-1}$) and T = the absolute temperature (°K) (Atkins 1989). This equation is only valid in dilute solutions.

As membranes are rarely permeable towards water molecules only, other chemical species may cross the membrane. The distribution of these species depend not only on the relative concentrations on each side of the membrane but also on their charge and the distribution of other charged species. It has been shown that positively and negatively charged species (i.e. cations and anions) will distribute to satiafy the Donnan equilibrium so that:

$$[Species^{+}]inside/[Species^{+}]outside = [Species^{-}]outside/[Species^{-}]inside$$

An important consequence of the Donnan equilibrium is that it may cause an unequal distribution of solute particles and thereby cause an osmotic pressure resulting in water movement.

In nature

Brackish water environments in Denmark consist of the littoral zones, estuaries and fjords. The majority of animals living in the brackish waters are euryhaline marine forms that have colonized the less saline environments (Rankin & Davenport 1981). Almost all marine invertebrates including most brackish water invertebrates are osmoconformers as opposed to osmoregulators which keep the osmotic pressure in their body fluids different from (most often higher than) that of the external medium (Schmidt-Nielsen 1990).

The advantage of being an osmoconformer is explained by the term itself. As the animal simply conforms to any external changes it does not waste energy, trying to uphold differences between the exterior and the interior. An osmoconformer adapted to a given salinity will have more or less the same osmolarity as the external medium (even though it is usually somewhat higher). Consequently, the are no net water fluxes due to osmotic pressure. When the organism faces a change in salinity, however, the volumes of both intracellular and extracellular water will change. It is then necessary for the organism to counteract this by changing the amounts of osmotically active substances. The prize paid for conforming is therefore that the internal milieu is subject to change.

Arenicola marina - an osmoconformer

Living in near-shore areas, *A. marina* is constantly exposed to fluctuations in the salinity of the ambient medium. The worm is a slightly hyperosmotic osmoconformer (Reitze & Schöttler 1989) and consequently has more or less the same internal osmotic pressure as the surrounding medium. This is consistent with the close relative *Abarenicola marina* that is slightly hyperosmotic at salinities between 10 and 33‰ (Oglesby 1973).

According to Robertson (1957), the concentrations of Na, Mg, Ca and Cl in coelomic fluid, expressed as percent dialysed against sea water, are all 100% except for K which is 104% and for SO_4 which is 92%. Also, the extracellular fluids contain no significant amounts of organic solutes (Robertson 1949). The ionic composition of the coelomic fluid is therefore virtually the same as for sea water. In contrast, the intracellular water has a K concentration which is higher and Na and Cl concentrations which are lower than in the extracellular fluids (Freeman & Shuttleworth 1985). Also, free amino acids contribute to almost half of the intracellular osmolytes (Reitze & Schöttler 1988). The ionic composition of the intracellular fluid is therefore very different from the composition of extracellular fluids and environment even though all fluids are isosmotic (Reitze et al. 1989).

The volume regulatory capacity of *A. marina* is based on both intracellular and extracellular mechanisms. The cellular water volume in the body-wall musculature is primarily regulated by the concentration of osmotically active free amino acids (Reitze & Schöttler 1989). Their concentration can be lowered by excretion, degradation or peptide anabolism. Apparently, degradation of glycine and D,L-alanine is a major mechanism of cellular volume regulation (Reitze & Schöttler 1989). Regardless of salinity the intracellular K^+ concentration is always kept high and Na^+ and Cl^- concentrations low compared to their concentrations in extracellular fluid (Freeman & Shuttleworth

1977, 1985). After transfer of lugworms from 34‰ salinity to 12‰ and thereafter back to 34‰ the restoration of cell volume was accompanied by active intracellular K^+ accumulation (Freeman & Shuttleworth 1985).

The regulatory mechanisms just described can not enterely prevent cells from swelling when worms are transferred to low salinity. Never the less, the swelling is only about 50% of what would have happended if amino acids were not lost (Freeman & Shuttleworth 1985).

During anaerobic conditions energy in body-wall musculature of *A. marina* is initially provided by degradation of phosphoryltaurocyamine, glycogen and aspartate. End products of anaerobic glycolysis are strombine and alanopine formed by combining pyruvate with glycine or alanine, respectively (Siegmund & Grieshaber 1985). When oxic conditions are restored alanine decreases and approaches normal levels within 2 hours (Pörtner et al. 1979). As lugworms are unable to degrade alanine during anaerobiosis, increased alanine concentration due to anaerobic glycolysis may influence the ability of the lugworm to regulate cell volume when exposed to reduced salinity. Indeed, Schöttler et al. (1990) found that lugworms kept under anoxic conditions reduced their metabolic activity and took up more water after transfer to low salinity than did lugworms treated similarly under normoxic conditions.

Extracellular fluids, mainly the coelomic fluid, is probably regulated via metanephridial excretion (Reitze 1987) and is described in more detail in V.

During stable external conditions, the lugworm would have no use for the ability to change its AWP (Apparent Water Permeability) as net water fluxes are negligible. However, during salinity changes and consequently a net inward or outward water flux, a capacity for decreasing permeability would be of advantage to the worm in order to restrain net water fluxes. Such an ability has been demonstarted in the polychaetes *Nereis diversicolor* and *N. limnicola* using D_2O (Smith 1964, 1970, 1976) or THO (Fletcher 1970, 1974) as a tracer. However, although *N. diversicolor* and *A. marina* are co-existing euryhaline intertidal polychaetes (Schöttler et al. 1990) it must be noted that *N. diversicolor* is considered an osmoregulator whereas *A. marina* is considered an osmoconformer (Oglesby 1975). Further, *Abarenicola pacifica* which is closely related to *A. marina* showed no reduction in permeability when adapted to low salinities (Oglesby 1978).

Bio-geochemical cycling of cadmium

The production and use of Cd increased during the eighties from 13.500 metric tonnes in 1982 to 16.500 metric tonnes in 1989. The production and uses of Cd have since decreased (Jensen & Markussen 1993). Never the less, Cd associated environmental problems have increased. Thus 8 times more Cd was consumed during the last 40 years than during the entire previous history of mankind (Stoeppler 1991).

Cadmium or its compounds are used in plastics, colour pigments, NiCd accumulators and in plating and alloys. Also, Cd is present as a component in fertilisers, chalk, coal, natural gasses, oil products and cement and as an impurity in zinc compounds (Jensen & Markussen 1993). Cadmium reaches the environment when the products mentioned above are used, burned, worn out, thrown away, deliberately spread on fields, etc. For 1990 it was estimated that 4.5% of the Cd released in Denmark reached the air, 1.6% the water, and 11.9% the soil. The remaining Cd would end up in garbage (68.9%) or be recycled (13.1%). However, these numbers are very uncertain (Jensen & Markussen 1993).

Coastal zones

Metals reaching the seawater originate mainly from rivers, the athmosphere or from hydrothermal inputs from active ridges. River inputs are to a large extent retained in estuaries and coastal zones (Salomons & Förstner 1984). It is estimated that 6340 kg of Cd reached Danish waters in 1990 and that 1020 kg originated from farmland, waste water and direct discharge whereas 4800 kg (approximately 78%) came from atmospheric deposition (Jensen & Markussen 1993). In 1982, Magnusson & Rasmussen reported the mean concentration of Cd in Danish coastal waters to be 25 $ng*l^{-1}$. The same concentration was found in danish streams in 1990 (Jensen 1991).

The water-sediment boundary

Sediments are know to constitute a concentrated pool of metals in aquatic environments (Luoma 1989), and Cd concentrations reaching 2.17 μg per g dry weight sediment have been reported in estuaries in the UK (Bryan & Langston 1992). A strong effect of *A. marina* on uptake and distribution of Cd in sediment was demonstrated in the present work (I,II). Also, bioturbating fauna promoted promotes the release of Cd from contaminated sediments of Foundry Cove, New York

State (Thomann et al. 1993), clearly indicating the effect of bioturbation on Cd fluxes between water and sediment.

The factors generally known to influence Cd binding in sediments (pH, oxidising/reducing conditions, etc), and thereby determine the distribution of Cd between sediment particles and pore water are discussed in more detail in I & II. However, a formula has been derived for estimating concentrations of toxicants in porewater at known sediment concentrations (Van Der Kooil et al. 1991). These are:

$C_{sed} = K_{sw} * C_w / r$ & $K_{sw} = K_{oc} * f_{oc}$

C_{sed} = Concentration in sediment (mg/kg).

K_{sw} = Solid-water partition coefficient (l/kg or l/g) (=130 for Cd).

C_w = Concentration in water phase (mg/l).

K_{oc} = Organic carbon partition coefficient (l/kg).

f_{oc} = fraction of organic carbon.

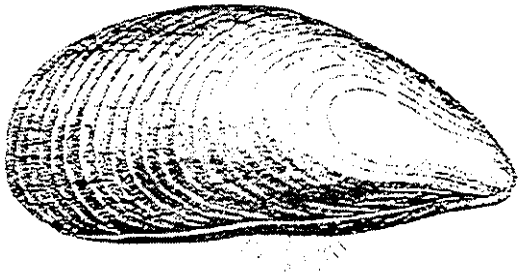
r = 1.5 for metals and 2 for organic pollutants.

Bioavailability of Cd

Cadmium in the benthic environment is available to the biota both as the free hydrated ion and as organic or inorganic complexes. Accumulation of Cd may occur as adsorption to outer surfaces and uptake across outer surfaces such as gills and intestine from both water and food (Rainbow 1988). It is generally assumed that the free hydrated ion is most important in relation to Cd bioavailability (Nugegoda & Rainbow 1989, Jenkins & Sanders 1986). The relative importance of Cd uptake from food as compared to water depends on several factors including metal concentration in food and water, the organism investigated and the metal in question (Luoma 1983, Bryan 1979).

In relation to Cd uptake via various exposure routes, sediment dwelling organisms such as the lugworm represent a special case. Due to the life style of the animal, it is difficult to discriminate between uptake from water and food. Luoma & Bryan (1982) and Ray et al. (1980) find that Cd uptake in *Nereis diversicolor* originates from interstitial water rather than from sediment particles. Similar results were also obtained by Ueda et al. (1976) for *Nereis japonica*. There are indications that this will be the case also for *A. marina* (Everaarts & SaralaDevi 1996) although other authors argue that food may be the most important Cd source (Selck et al. 1998). However, it must be noted that the bioavailability of metals in sediment appears to depend on the geochemistry of the sediment

(Luoma 1989). A review by Di Toro et al. (1991) established that although there are no relationships between sediment chemical concentration on a dry weight basis and biological effects there is a good correlation between chemical concentration in the sediment porewater and biological effects. It is speculated, that the sediment-porewater equilibrium system provides the same exposure as water-only exposure (Di Toro et al. 1991). A further complication is that *A. marina* actively participates in changing its surroundings. The bioturbating activity of the lugworm results in differences between the sorrounding sediments and the lugworm burrow where sediment is oxygenated and pH is high resulting in binding of Cd to Mn and Fe oxides and hydroxides (Simpson 1981, Förstner & Wittmann 1979). The lugworm feeds on the sediment and consequently Cd bound to sediment particles will reach the intestine of the worm. Deposit feeders may take up molecules in the lumen by alkaline refluxing and the intestinal pH is then consequently not low but high (Lopez & Levinton 1987).



Mytilus edulis

A common mollusc at St. Havelse

List of Papers, Research reports & Presentations

The thesis is based on the following papers and research reports

I *Rasmussen AD, Banta GT, Andersen O (1998) Effects of bioturbation by the lugworm Arenicola marina on cadmium uptake and distribution in sandy sediments* ***(Marine Ecology Progress Series 164,179-188).***

II *Rasmussen AD, Banta GT, Andersen O. Cadmium dynamics in estuarine sediments - effects of salinity and lugworm bioturbation* ***(Environmental Toxicology & Chemistry, submitted).***

III *Rasmussen AD, Andersen O. Cadmium transfer between water and sediments studied in laboratory experiments and with a computer model* ***(Bulletin of Environmental Contamination and Toxicology, submitted).***

IV *Rasmussen AD, Banta GT, Andersen O. Population analysis, allometric relations and bioturbation activity of Arenicola marina in a Danish Fjord* ***(Ophelia, to be submitted).***

V *Rasmussen AD, Andersen O. Effects of cadmium exposure on volume regulation in the lugworm, Arenicola marina* ***(Aquatic Toxicology, submitted).***

VI *Rasmussen AD, Andersen O. Volume regulation in the lugworm Arenicola marina during exposure to cadmium or hypoxia* ***(research report).***

As well as on the method described in the paper added as an appendix:

VII *Rasmussen AD, Andersen O (1996) Apparent water permeability as a physiological parameter in crustaceans* ***(J exp Biol 199,2555-2564).***

Parts of the data have been presented or will be presented at the following meetings

- Rasmussen AD. Biomarkers in *Arenicola marina*. **Oral presentation**, 1. Science Symposium Day, RUC, 1995.
- Rasmussen AD, Andersen O. Effects of heavy metals on volume regulation in *Arenicola marina* exposed to low salinities. **Poster presentation**, SETAC'97, Amsterdam, 1997.
- Rasmussen AD, Banta GT, Andersen O. Effects of bioturbation by the lugworm on uptake and distribution of cadmium in sediment. **Oral presentation**, SETAC'97, Amsterdam, 1997.

- Rasmussen AD, Andersen O. Adaptations to a changing environment; Effects of stressors on osmoregulatory capabilities. **Oral presentation**, Science Day 1997, RUC, 1997.
- Rasmussen AD, Andersen O. Effects of cadmium exposure on adaptational capacity to osmotic stress in *Arenicola marina* (Polychaeta) and *Carcinus maenas* (Decapoda). **Poster presentation**, Studying stress in ecological systems: Pure and applied perspectives, RUC, 1997.
- Rasmussen AD, Andersen O. Effekter af cadmium på osmo- og volumenregulering hos estuarine invertebrater. **Oral presentation**, Det 10. Danske Havforskermøde, Hirtshals, 1998.
- Rasmussen AD, Banta GT, Andersen O. Sandorms bioturbation ændrer optagelse og fordeling af cadmium i sediment. **Poster presentation**, Det 10. danske havforskermøde, Hirtshals, 1998.
- Rasmussen AD, Banta GT, Andersen O. Effects of bioturbation by the lugworm on uptake and distribution of cadmium in sediment. **Oral presentation**, Friday seminars, RUC, 1998.
- Banta GT, Conley DJ, Rasmussen AD, Timmerman K, Andersen O. Bioturbation by the lugworm *Arenicola marina* - Implications for transport and distribution of materials in tidal flats. **Poster presentation**, Organism-sediment interaction workshop, Georgetown, South Carolina, 1998.
- Banta GT, Forbes T, Kure L, Rasmussen AD, Timmermann K, Andersen O. Attempt to reduce uncertainty in risk assessment for polluted sediment: Development of a mechanistic linked fate and transport model of infauna-toxicant mutual interactions. **Oral presentation**, SETAC'99, Leipzig, Germany, 1999.
- Rasmussen AD, Banta GT, Andersen O. Lugworm bioturbation affects cadmium water/sediment transfer and distribution. **Oral presentation**, ASLO, Santa Fe, New Mexico, 1999.
- Banta GT, Timmerman K, Rasmussen AD, Andersen O. Pollutant effects on bioturbation - an added wrinkle in interactions between geochemistry and the benthos. **Oral presentation**, ASLO, Santa Fe, New Mexico, 1999.
- Rasmussen AD, Andersen O. Modelling studies on factors affecting sediment-water Cd transfer in estuaries. **Poster presentation**, ASLO, Santa Fe, New Mexico, 1999.

Results & discussion

The results of the separate investigations are discussed in the corresponding articles. This section compare and discuss the results in general thereby hopefully putting them into perspective to answer the following overall questions: which abiotic factors determine the fate of Cd in an estuarine environment, how is the fate affected by bioturbating organisms, in particular *Arenicola marina* and, how does Cd toxicity affect the estuarine infauna?

1. Transfer and distribution of Cd between water and sediment

1.1 Effects of bioturbation

Three approaches were used to study effects of bioturbation on Cd distribution between water and sediments: daily additions of Cd to the water phase (I); a single addition of a thin layer of Cd labelled sediment to the sediment surface, and (II); homogeneously labelling of the entire sediment column with Cd (II).

In sediments without lugworms water-borne Cd accumulated in the top sediment layers and penetrated only slowly into deeper sediment layers. The presence of lugworms changed this picture as the lugworm's irrigational water current through the entire burrow, dramatically increased the area of the sediment-water interface. This resulted in faster removal of Cd from the water phase and in rapid buildup of Cd peaks at the feeding depth of the worm. Sediment turnover also affected the Cd distribution. Fecal casts on the sediment surface, as well as the uncovering of previously unexposed sediment, aided in increasing the area of the sediment-water interface. Further, the fecal

casts covering the Cd polluted top sediment caused a downward movement of Cd bound to sediment. The two components of bioturbation, solute and particle movement, thus acted synergistically in enhancing sediment uptake of Cd as was also seen in a small *in situ* investigation in Roskilde fjord (IV), eventually leading to a more or less homogeneous Cd distribution through the sediment different from the distribution in sediment without worms. The general effect of bioturbation on Cd distribution has also been described by Everaarts & SaralaDevi 1996 and Thomann et al. 1993.

Addition of Cd as a thin layer on the sediment surface gave results corresponding to the above: The Cd profiles of sediments without worms remained almost unaltered throughout the experimental period while the presence of worms strongly affected the vertical Cd distribution. Cadmium peaks arose at the feeding depth indicating that the Cd released to the water phase was transported downward by irrigation. Also, the Cd-spiked top sediment layer was soon buried by fecal casts resulting in an increasingly homogeneous Cd distribution. When sediment was labelled uniformly with Cd (II), however, bioturbation resulted in a movement of Cd from depth to surface to a degree not seen when worms were absent. Bioturbation may further promote Cd release as has been observed by Thomann et al. (1993) and Emerson et al. (1984). The actual release may, however, be an indirect effect as sediment oxidised due to bioturbation to some extent releases Cd (Lu & Chen 1977) and bioturbation constantly exposes previously "hidden" anoxic sediment to oxidising conditions, both by irrigation and by transport to the sediment surface. Enhanced Cd release due to irrigation was not observed in the present experiments, probably because of the experimental set-up that did not allow continuos water change in the sediment cores and because the sediments have a high capacity for binding dissolved Cd.

1.2 Effects of salinity

One main component of brackish and seawater is the Cl^- ion, the ion primarily responsible for Cd speciation due to the formation of Cd-chloro complexes (III, Kester 1986). In short term experiments studying effects of the speciation of Cd on the transfer of ^{109}Cd from water to sediment, transfer rates and fractional amounts of Cd^{2+} correlated significantly (III), suggesting that, as expected (Stumm 1992), the hydrated Cd^{2+} ion itself was the most particle-reactive species. Furthermore, the release of Cd from sediment to water increased with increasing salinity indicating

that the binding of Cd to sediment particles is reversible but with an equilibrium shifted strongly towards sediment binding of Cd.

When both salinity and bioturbation were investigated simultaneously in sediment cores (II) the effect of salinity on Cd flux was much less pronounced than seen in III. This was probably due to both experimental set-up and the more complex situation: First, Cd release was measured for 21 days in II instead of during 24 hours as in III. Second, in II Cd could diffuse into lower sediment layers thus changing Cd release. Third, sediment particles and Cd-particle interactions could have changed due to ageing of the sediment (Salomons & Förstner 1981). And, fourth, irrigation moved overlying water into the lugworm burrow in II thus decreasing contact with the overlying water. Never the less, as would be expected Cd release was highest at 24‰ salinity due to increased formation of Cd-chloro complexes (Davies-Colley et al 1984).

1.3 Other parameters affecting Cd distribution

The Minteqa2 Cd speciation model revealed that while salinity was the main factor controlling Cd speciation in seawater, pH strongly affected Cd speciation in freshwater (III, Kester 1986). In freshwater the fraction of Cd^{2+} decreased with increasing pH from 80% at pH 7 to 10% at pH 9, mainly due to formation of $CdCO_3$. Seawater is well buffered and Cd speciation is thus normally less influenced due to the small pH changes. Neither temperature nor Cd concentration affected Cd speciation.

Laboratory experiments indicated that the ratio between water volume and sediment surface area affected the Cd transfer rates both to and from sediments, as did the Cd concentration itself (III). This confirmed the results of a previous investigation (I) where the ratio between water volume and sediment surface area had increased due to the lugworm burrow. It may then be hypothesised that the water residence time in a near shore area will affect the total transfer of Cd from water to sediment. The longer residence time, the more of the dissolved Cd has the potential to reach sediment particle surfaces. The total Cd flux from water to sediment depend on the flux rates described above that in tern dependent on external factors and on the actual Cd concentration in the overlying water. This may indeed be the most important parameter, especially in highly polluted areas, as sediments are able to take up large quantities of Cd from water and thereby act as a "Cd sink" (I, Bryan & Langston 1992). Aging of sediments results in altered and thereby enhanced binding between Cd and sediment particles (Salomons & Förstner 1984). This effect was not

evident, however, in sediments aged for 1.5 months before the release of Cd to water was estimated (III). Finally it may be mentioned that my unpublished results suggested that other sediment parameters (e.g. organic content) also affected the uptake of Cd as previously reported by Stumm 1992.

1.4 Distribution of Cd between water, sediment and infauna

The pore water Cd concentration was almost undetectable (I) and, even though up to 30% of the sediment volume could be pore water, the total amount of Cd found in this fraction was thus very low. In contrast, the sediment bound Cd fraction was very high. As sediment particles constituted more than 70% of the entire sediment columns (I, II), virtually all Cd was found present in sediment. In cores containing 25 cm of sediment and a lugworm density of 190 worms/m^2, which is very high (IV), 1-3% of total Cd was found in worms (I). However, as the total lugworm biomass was only approximately 0.1% the lugworm Cd bioconcentration factor could be estimated to be between 10 and 30. The highest Cd concentrations in the experimental system were thus found in lugworms that are known to accumulate Cd from sediments (Bryan & Gibbs 1987).

2. Cadmium uptake and effects on the lugworm

2.1 Uptake and distribution of Cd in Arenicola marina

Several experiments indicated that Cd added to the water phase was taken up into lugworms, regardless of whether these were kept in sediment or in seawater only (I, II, V, VI). The largest amounts of Cd were always found in the body-wall, the chloragogenous tissue and oesophagal tissue while the Cd concentration of the coelomic fluid was very low except during the first hours of exposure. This agrees with the fact that the coelomic fluid has almost no organic ligands (Freeman & Schuttleworth 1977) and consequently few binding sites. This pattern was seen also in worms exposed to Cd added directly to the sediment (II).

How sediment dwelling polychaetes take up Cd is still not known. Several experiments indicate that Cd accumulation in annelids occurs mainly as uptake of the free hydrated Cd^{2+} ion from interstitial water (Mason et al. 1988, Jenkins & Sanders 1986, Ray et al. 1980). That Cd uptake in some cases correlated better with porewater Cd concentration than with total sediment Cd concentration can, however, also mean that the animals are extracting Cd from sediment fractions which equilibrate quickly with porewater (Rule & Alden 1996). As discussed in the previous section, lugworm bioturbation may promote the release of Cd from contaminated sediments back to the overlying water. Thus, as the worm pumps the overlying water through it's burrow it may incerase it´s own Cd exposure. The release of particle reactive substances e.g. oil components from sediment to irrigation water has been observed for other conveyor belt feeders (Reible et al. 1996, Koerting-Walker & Buck 1989).

Even though our experiments were not designed to investigate the pathways by which Cd was taken up by and distributed in lugworms, some information can be obtained by comparing uptake and distribution in experiments where various set-ups were used. The data in Table 1 suggest that worms kept sediment free or exposed by irrigation initially take up/adsorb Cd to the body-wall due to contact with Cd-laden water (V, I). This fast initial uptake has previously been described in lugworms (Everaarts & SaralaDevi 1996). After exposure for longer time periods, however, the Cd concentration of the chloragogenous tissue exceeds the concentration in body-wall tissue. This could be caused by an internal transport of Cd from the body-wall to the chloragogenous tissue that functions as a storage organ (Krüger 1971). Such a transport is evident in the crab *Carcinus maenas* (Bjerregaard 1991). It could, however, also be the result of altered exposure routes: sediment vs. porewater vs. overlying water. In the two cases where the ratio of Cd concentrations between body-

wall and chloragogenous tissue are less than 1 (Table 1) worms had been feeding on Cd contaminated sediment particles for some time. When exposed to Cd uniformly distributed in the sediment, the polychaete *Capitella* sp. 1 took up Cd mainly from the sediment bound fraction (Selck et al. 1998). The dominating pathway of Cd uptake thus seems to depend on how Cd is presented to the worm in the experimental system and therefore perhaps how Cd reaches and is distributed in a near shore area.

Table 1: Cadmium concentration in body-wall vs. chloragogenous tissue.

Paper	Exposure time	Cd added to	System set-up	Ratio of Cd concentration
V	54 hours	Overlying water	Water	≈ 10
V	1 month	Overlying water	Sediment + water	≈ 0.8
II	21 days	Sediment top	Sediment + water	≈ 1.2
II	21 days	Whole sediment column	Sediment + water	≈ 0.4

Finally, it should be mentioned that when Cd was added to the water phase lugworms took up Cd almost linearly during 16 days of exposure (I). Also, when exposed to 1 ppm Cd by water only, worms took up approximately 10 times more Cd that worms exposed to 0.1 ppm Cd (V). When exposed to Cd added to the sediment, however, Cd uptake did not correlate well with time of exposure. The apparent differences in Cd accumulation during different exposure situations calls for a good understanding of uptake mechanisms and carefull interpretation of data if *A. marina* is to be used as a biomonitoring organism. Bryan and Gibbs (1987) have previously coined *A. marina* a moderate indicator of sediment Cd availability.

2.2 Volume regulation; Effects of stressors

The lugworm´s osmoadaptational capacity via volume regulation was very effective, as worms exposed to reduced external salinity obtained new weight equilibria close to the original weights within a short period of time and as mortality was low (V). It is thought that volume regulation is accomplished mainly extracellularly by urinary excretion of excess water (Reitze 1987), while cell volume is allowed to increase (Reitze & Schöttler 1989), in agreement with the results presented here. Exposure to Cd (V) or anoxia (VI), however, markedly reduced the ability of the worms to successfully adapt to lower salinities. Apparently this was due to interference with the extracellular volume regulation and caused increased mortality. This result is of general interest as toxicity

testing in most instances is performed during constant abiotic conditions thus keeping natural stress at a minimum. Another example of increased Cd toxicity combined with "natural" stress was observed in starving *Capitella* sp. 1 (Selck et al. 1998).

How Cd inhibits volume regulation is not known, but an interaction with the nephridial function is likely. Worms exposed to Cd for 1 month at constant salinity also had larger volumes of coelomic fluid compared to control animals (V). As the worms are slightly hyperosmotic, this could have been caused by impaired nephridial function. Also, Cd is known to accumulate in the kidney-like tissues of marine bivalves, gastropods and crustaceans (Nott 1991, Eisler 1981). It is not known whether Cd may affect AWP during salinity changes (see discussion 2.3 below).

Transfer of lugworms to higher external salinitiy caused worm weights to decrease due to osmotically driven water loss (opposite direction as described above). Again, Cd exposure resulted in worms having higher weights than control worms. This result is somewhat difficult to explain and needs further investigations (See VI). As almost no work has been done on osmoconforming polychaetes transferred to higher salinities, comparable data are not available. Oglesby (1978), however, generally described worm water loss as a mirror image of the effect of hypo-osmotic transfer even though water efflux is less rapid than influx. This agrees with our results (VI). Furthermore, Cd exposure in combination with hyper-osmotic transfer does not cause increased mortality. This confirms data from several authors (compiled by Oglesby, 1978) indicating that while transfer to low salinity may cause mortality transfer to high salinity usually is harmless.

2.3 Apparent water permeability; Effects of stressors

While Cd exposure affected the AWP of several hyper-regulating crustaceans (VII) neither Cd nor anoxia affected the AWP of the osmoconforming lugworm (V, VI). Salinity changes also do not affect AWP in *Abarenicola marina* (Oglesby 1978) suggesting that AWP changes are not employed as an osmoregulatory tool in the osmoconforming lugworm species (*Arenicola* spp. & *Abarenicola* spp.). In crustaceans, where AWP has been thoroughly investigated, changes are thought to be effected by the gills as the cuticula is virtually impermeable to water (VII). In lugworms, however, such a regulation would not be possible due to the water permeability of the entire body-wall. Never the less, various hyper-regulating polychaete species are able to lower their AWP when exposed to dilute media (Smith 1976), indicating that it is the osmoconforming strategy itself that makes control of the AWP superfluous. Whereas hyper-regulating organisms would experience a benefit of AWP

control even at invariable external salinity due to the constant influx of water, osmoconformers would benefit only during salinity changes. The general AWP method, however, allows AWP calculations only when net water fluxes are low (VII), so if lugworms utilise short term AWP changes during salinity changes this would be difficult to measure.

2.4 Bioturbation; Effects of stressors

Exposure to 1 ppm Cd did not seem to affect neither the solute nor the particle mixing part of the lugworms bioturbation (I). Results indicate, however, that bioturbation halts when worms are exposed to 10 ppm Cd (V) and that this concentration leads to the eventual death of the worms. It therefore seems as if the effects of Cd exposure on bioturbation is an all-or-non response or a very steep dose-response correlation. Also, salinities within the range 12 to 24‰ did not affect irrigation (II) measured as Br^- flux (Aller & Aller 1992). All observations were made after worms had been exposed to Cd or a given salinity for some time (days), so it is not known which initial effects Cd exposure or salinity changes may have on bioturbation. Overall, bioturbation by the lugworm was not affected by Cd exposure, except at high Cd concentrations (10 ppm). More data would be needed to establish good dose-responce relations.

2.5 Concluding remarks

Short term salinity changes combined with exposure to Cd (V, VI) or anoxia (VI) all resulted in effect on volume regulation as did long term Cd exposure (V). Long term Cd exposure or exposure to various salinities did not affect bioturbation (or perhaps AWP), however. Long term exposure to anoxia will affect irrigation as a main function of irrigation is to pump oxygenated water past the worms gill filaments (Krüger 1971). These observations may be explained by the fact that *A. marina* is an osmoconforming species living in a variable environment and that it's strategy is to adapt to the environment. If the effects of salinity changes or Cd exposure on bioturbation occur only briefly, they could not be observed in the present long term experiments.

3. Conclusions

The results of the present investigations (I - IV) confirm that sediments are a sink for water borne Cd. While not evident in the present results, it has been also suggested that the before mentioned "Cd sink" may turn into a "Cd source" due to the pathways of Cd uptake into polychaetes (Ray et al. 1980). Also, the constant vertical mixing of sediments may promote the release of Cd back to water in areas where sediment is contaminated (Thomann et al. 1993). The extent of this mixing (both depth and speed) depends on the density of bioturbating organisms. In the case of lugworms, the distribution may be very patchy although their density decreases with increasing distance to the shore line (IV). In such environments, the bulk partitioning of Cd between water and sediment will be difficult to assess as it depends on lugworm bioturbation and ultimately density. According to Reible et al. (1996), species with conveyor-belt feeding modes, such as the lugworm, will perhaps have the largest impact on the partitioning of metal contaminants between the water and the solid phase. The results presented here suggest that they cause a net Cd flux from water to sediment in almost all cases.

Consequently, the fate of Cd reaching an estuarine system depends on both biotic and abiotic factors that vary spatially and with time in the estuary. A constant low concentration of Cd in the water phase will likely result in high sediment Cd concentrations that eventually may be in equilibrium with the water phase, at least where sediments are bioturbated. Organisms living in such areas are likely to be continuously exposed to Cd, even if the Cd contamination ceases, as the sediments may release Cd as has been calculated for Foundry Cove (Thomann et al. 1993). Accordingly, a short term release of Cd to an estuary will be buffered as most Cd will adhere to sediment particles and hence lower the exposure by water. The result could thus be a more constant exposure of infauna to Cd in sediments and pore water and to organisms living above sediment due to sediment Cd release.

The natural estuarine variations in salinity both directly and indirectly affect all parameters investigated in this thesis. Cadmium speciation is strongly salinity dependent, and speciation further influences Cd uptake in sediment and biota. The uptake of Cd also interferes with the ability of the biota to handle salinity stress. Sediment uptake of Cd is also affected by infaunal biota and will in turn affect infaunal Cd exposure on a long term basis as Cd may be "locked safely away" or may be rendered more bioavailable to the biota. This very complex scenario makes it difficult to assess the fate and effect of Cd pollution of estuarine areas. More work, which takes the inherent variability of

estuarine environments into account, must be done to further an understanding of the factors regulating the fate and effects of pollutants in such dynamic ecosystems.

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I

EFFECTS OF BIOTURBATION BY THE LUGWORM *ARENICOLA MARINA* ON CADMIUM UPTAKE AND DISTRIBUTION IN SANDY SEDIMENTS

Rasmussen AD, Banta GT, Andersen O

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Effects of bioturbation by the lugworm *Arenicola marina* on cadmium uptake and distribution in sandy sediments

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ABSTRACT: The effect of bioturbation by the lugworm *Arenicola marina* on uptake and distribution of cadmium in sediment was assessed using laboratory sediment cores. Carrier-free ^{109}Cd was added to the water phase each day. Bioturbation (irrigation) was measured using bromide (Br^-) as a tracer for water movement. In cores without lugworms all Cd was found in the surface sediment where it continued to build up over 16 d of exposure. In cores containing lugworms a distinct peak of Cd was found both at the sediment surface and, after a few days, at the feeding pocket of the worm (10 to 15 cm depth). During the 16 d of exposure this subsurface peak broadened and eventually Cd was found in all depths from top to feeding depth of the individual worm. Compared to sediment cores without worms, the presence of lugworms more than doubled the rate of removal of Cd from water to sediment. This was attributed to an increased turnover of sediment (due to feeding activity), an increased sediment surface area (due to fecal casts, head shaft, tube and irrigation of the whole burrow) and an increased contact of Cd-labelled water with potential binding sites in the sediment due to irrigation. Exposure to 1 ppm Cd reduced the fractional rate at which lugworms removed Cd from the water (as % of Cd in the water). The total Cd flux to the sediment, however, was much greater due to the higher Cd concentrations in the water. Water fluxes estimated using Br^- as a solute tracer revealed a 10- to 20-fold increase in water exchange of the sediments when lugworms were present. This enhanced water flux was not affected by exposure of lugworms to 1 ppm Cd. The results indicate that the presence of bioturbating infauna influences both the uptake rates of trace metals in near-shore sediments and the distribution of those metals.

KEY WORDS: *Arenicola marina* · Cadmium · Irrigation · Bioturbation · Sediments

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II

CADMIUM DYNAMICS IN ESTUARINE SEDIMENTS - EFFECTS OF SALINITY AND LUGWORM BIOTURBATION

Rasmussen AD, Banta GT, Andersen O

Environ Toxicol Chem, submitted

Cadmium dynamics in estuarine sediments - effects of salinity and lugworm bioturbation

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ABSTRACT

We investigated the effects of lugworm bioturbation on the fate of Cd added either to a thin layer at the sediment surface or homogeneously mixed throughout the sediment. In both situations, the Cd release to the overlying water was highest when lugworms were not present, most likely because bioturbation transported Cd-contaminated sediment away from the sediment surface. Also, irrigation transported water-borne Cd back into the sediment.

When Cd was added to the sediment surface, a Cd peak emerged at the feeding depth of the worm within 1 day due to the transport of water-borne Cd down into the sediment by lugworm irrigation. In addition, the conveyor-belt feeding mode of the worm caused both Cd to be buried by fecal casts and a gradual spreading of the Cd distribution within the sediment column. When Cd was added to the entire sediment column, bioturbation caused a net transport of Cd upwards resulting in the surface layers having higher Cd concentrations than the deeper layers indicating a net release of Cd from deeper sediments.

The distribution of Cd in lugworms depended on the Cd exposure situation and suggested that worms were exposed mainly to water-borne Cd when Cd was added to the top of the sediment, while worms were exposed mainly by ingesting Cd labelled sediment when Cd was mixed homogeneously throughout the sediment.

Keywords: Bioturbation; Cadmium; Salinity; *Arenicola marina*; Metal speciation;

III

CADMIUM TRANSFER BETWEEN WATER AND SEDIMENTS STUDIED IN LABORATORY EXPERIMENTS AND WITH A COMPUTE MODEL

Rasmussen AD, Andersen O

Bull Environ Contam Toxicol, submitted

Cadmium Transfer between Water and Sediments studied in Laboratory Experiments and with a Computer Model

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The transfer of Cd between water and sediment is a complex process controlled by factors such as water salinity, pH and content of humic substances as well as sediment composition, redox properties and oxygenation (Luoma 1989, Davies-Colley *et al.* 1984, Lu & Chen 1977). Frequent variations in the above mentioned abiotic parameters occur in estuaries due to tidal changes and fresh water run off. Further, fresh water run off may be polluted with trace metals. A general accumulation of pollutants is believed to occur in estuarine sediments and Cd concentrations as high as 130 mg/kg dry weight sediment have been found in UK estuaries (Bryan & Langston 1992). Several parameters such as water residence time, metal concentration, salinity and bioturbation by sediment dwelling organisms could potentially influence the partitioning of metals between water and sediment (Rasmussen *et al.* 1998). For instance, in fresh water more than 70% of the total Cd is in the form of Cd^{2+}. The amount of this ionic form decreases rapidly with increasing salinity due to the formation of Cd-chloro complexes (Kester 1986, Mantoura *et al.* 1978), probably resulting in decreases adhesion of Cd to sediment particles. When salinity is increasing, the higher water concentrations of Ca^{2+} and Mg^{2+} will exchange with sediment bound Cd. Even though sediments usually contain low fractions of organic material, the affinity of this fraction for Cd is high due to the presence of functional groups such as -COOH, -OH and -SH (Stumm 1992). The affinity is believed to be independent of pH and redox processes (Davies-Coley *et al.* 1984). During oxidising conditions, Cd adheres to Fe and Mn oxides and hydroxides that are part of the inorganic fraction of the sediment. During reducing conditions, Fe and Mn bound to sulphides will exchange with Cd whereby mainly Cd monosulphides of very low solubility is formed (Di Toro *et al.* 1990). These insoluble complexes are also known as AVS (acid-volatile sulphides) (Pesch *et al.* 1995).

IV

POPULATION ANALYSIS, ALLOMETRIC RELATIONS AND BIOTURBATION ACTIVITY OF *ARENICOLA MARINA* IN A DANISH FJORD

Rasmussen AD, Banta GT, Andersen O

Ophelia, to be submitted

Population analysis, allometric relations and bioturbation activity of *Arenicola marina* in a Danish Fjord

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Keywords: Population, *Arenicola marina*, Lugworm, Bioturbation, Allometry.

Running head: Population analysis of Arenicola marina.

Abstract

In Roskilde fjord, Denmark, a population of lugworms (*Arenicola marina*) was investigated along a transect ranging from the shore line to the shipping channel. Water depth increased slowly from 0 m at the shore line to approximately 1.5 m at a distance of 325 m from the shore line after which the depth increased rapidly at the start of the shipping channel which is more than 10 m deep.

Data on lugworm density, size and estimates of sediment reworking and irrigation suggested that the transect could be divided into three zones: one close to the shore where both sediment reworking and irrigation were intense, up to 1,300 ml sediment/m^2/day and 22 L seawater/m^2/day, respectively, although not reaching deep into the sediment; a second zone ranging from 25 m to approximately 100 m off shore where sediment reworking was intense and deep but where irrigation activity was decreasing; and a third zone from 100 m and further out with almost no sediment reworking or irrigation as worms were virtually absent.

Lugworm size increased with increasing distance to the shore. Allometric data showed that chloragogenous and body wall tissue constituted the same amount of total worm weight in adult worms (ranging from below 1 to above 8 g). Never the less, the relative tail length was significantly larger in large worms, perhaps as an adaptation to the deeper burrows. Also, a significantly higher portion of large worms were dark, but colour did not correlate significantly with skin storage of the respiratory pigment breakdown product biliverdin.

V

EFFECTS OF CADMIUM EXPOSURE ON VOLUME REGULATION IN THE LUGWORM, *ARENICOLA MARINA*

Rasmussen AD, Andersen O

Aquat Toxicol, submitted

Effects of cadmium exposure on volume regulation in the lugworm, Arenicola marina

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Abstract

Effects of hypo-osmotic stress or Cd (cadmium) exposure stress, applied singly or in combination, on volume regulation were investigated in A. marina., the common European lugworm. In short term experiments, the combined exposure to Cd and hypo-osmotic stress mainly affected the worm's capacity for regulating the coelomic fluid volume without significantly affecting the regulation of intracellular fluid volume. Exposure to Cd increased the worm's sensitivity to hypo-osmotic stress noted as increased mortality compared to the mortality in groups exposed only to Cd or hypo-osmotic stress. In long term Cd exposure experiments, the capacity for coelomic fluid volume regulation was affected even at constant external osmolarity and the tissue water concentration decreased slightly. The results could not be explained by changes in AWP as Cd did not significantly affect this. The glycogen content of the chloragogenous tissue did, however, increase during long term Cd exposure suggesting metabolic stress.

The results demonstrate, that two stressors, each without serious effects when applied alone, may have fatal effects when applied in combination. The kind of osmotic stress applied in these experiments is common in many biotopes inhabited by the lugworm. The severe effects of the combination with Cd stress indicate, that the classical ecotoxicology testing could conceivably underestimate the toxicity of chemical compounds due to not considering combined effects of natural and anthropogenic stressors.

Key words

Volume regulation; Cadmium; Polychaeta; Salinity changes; Apparent Water Permeability; Mortality.

VI

VOLUME REGULATION IN THE LUGWORM *ARENICOLA MARINA* DURING EXPOSURE TO CADMIUM OR HYPOXIA

Rasmussen AD, Andersen O

Research report

Volume regulation in the lugworm *Arenicola marina* during exposure to cadmium or hypoxia

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Abstract

Volume regulation in *A. marina* exposed to hyper-osmotic media was found to be a "mirror image" of the volume regulation in worms exposed to hypo-osmotic media even though total water fluxes were lower. Exposure to Cd prior to salinity transfer altered the volume regulation causing exposed worms to return to a weight closer to the initial weight than the control worms. This effect awaits further clarification.

Lugworms exposed to anoxia prior to transfer to lower salinity took up more water and returned slower towards the pre-shift weight than did control worms. Never the less, the apparent water permeability was unaffected by anoxia and could not explain the anoxia-induced increase in water uptake compared to that in control animals. Anoxia thus likely affects the metanephridial excretion of urine formed by coelomic fluid. Lugworms kept at low oxygen concentrations (40 or 0% normoxia) at 15‰ salinity had a tendency to take up more water than worms kept at normoxia.

Whereas combined exposure to Cd and hypo-osmotic media generally increased lugworm mortality this was not the case when Cd-exposed worms were transferred to hyper-osmotic media. This could be because even short periods of increased cell volume may result in cell rupture and thus cell death while a decreasing cell volume is less harmful.

Appendix

VII

APPARENT WATER PERMEABILITY AS A PHYSIOLOGICAL PARAMETER IN CRUSTACEANS

Rasmussen AD, Andersen O

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REVIEW

APPARENT WATER PERMEABILITY AS A PHYSIOLOGICAL PARAMETER IN CRUSTACEANS

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Summary

This article reviews the use of apparent water permeability (AWP) calculated from measurements of isotope-labelled water flux as a physiological estimate of whole-body water permeability in aquatic invertebrates. The rationale and practices of AWP calculations are described in an Appendix.

AWP calculations have provided a wealth of information. However, the validity of the method and therefore also of the information obtained have been questioned. Consequently, the use of AWP data in discussions of osmotic and fluid homeostatic questions in aquatic invertebrates is limited. This article reviews three decades of published experiments in which measurements of isotope-labelled water fluxes were used to estimate water permeability in aquatic invertebrates. Data on 24 species of arthropod, most of them decapod crustaceans, are presented. The combined data indicate that the results obtained by different investigators on the same species show good agreement, even though different tracers and experimental methods have been applied. When available, results from other kinds of studies were used to evaluate the results obtained using the AWP measurements. The various results demonstrate that AWP is influenced not only by natural environmental factors, such as salinity and temperature, and by anthropogenic factors, such as potentially toxic trace metals, but that it is also regulated by intrinsic factors, such as ecdysis and life cycle stage. The results obtained can often be explained as effects of components of the habitat of the animal. Accordingly, studies on variations in AWP contribute to our understanding of the different physiological strategies used by species living in a changing environment.

We conclude that calculations of AWP offer reliable, relevant physiological data in a range of crustacean species, as long as methodological limitations and uncertainties are kept in mind.

In addition, we propose some possible new ways of applying AWP calculations to marine invertebrates other than crustaceans. A major part of this review describes results already obtained for the shore crab *Carcinus maenas* as this species is probably the animal on which most work has been carried out. We suggest topics for future work on this species and review the possibility of using AWP in *C. maenas* as a biomarker of metal exposure.

Key words: aquatic invertebrates, osmoregulation, trace metals, salinity, ecdysis, individuality, *Carcinus maenas*, metal uptake, isotope flux.

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